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Full Length Research Paper

Bio-activity of oils of *Trigonella foenum-graecum* and *Pongamia pinnata*

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Different concentration of oils obtained from two plants species belonging to family Fabaceae i.e. *Trigonella foenum-graecum* and *Pongamia pinnata* were evaluated for their antifungal and antibacterial activity against *Aspergillus niger, A. fumigatus, Staphylococcus aureus* and *Pseudomonas aeruginosa* by MIC determination and dry-weight method. Both the oils showed high degree of antimycotic and antibacterial activity. *P. pinnata* oil was more effective as compared to oil of *T. foenum-gracecum*. *A. niger* and *S. aureus* were more sensitive to oil of *P. pinnata.* Chemical analysis of oils performed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) showed the presence of fatty acids.

Key words: Inhibition, human pathogenic microbes, oils, *Trigonella foenum-graecum, Pongamia pinnata*.

INTRODUCTION

In recent years, there has been gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries, because plant derived drugs have been reported to be safe and without side-effects (Pandey and Rai, 2003). Thus, there is a pressing need for new plants-based drugs (Abdo and Al-Kafawi, 1969; Chatterjee and Parkashi, 1995; Singh, 1999; Brunaton, 1999; Pederson et al., 1999; Singh, 1999; Simin et al., 2000; Dubey et al., 2000; Kapoor, 2001; Yoshiikazu et al., 2001; Rai et al., 2004; Romagnoli et al., 2005). Very few antifungal substances are available in the market when compared to antibacterial substances, and they are also relatively unsatisfactory in controlling the diseases caused by fungal infections. The problem of drug resistance in microbes is increasing day by day (Gold and Moellering, 1996; Teshager et al., 2000; Cohen, 2001; WHO, 2001).

A perusal of the literature indicates that many investigators have reported fungistatic and bacteriostatic properties of extracts of higher plants (Chopra et al., 1969; Jain and Agarwal, 1976; Daphne et al., 1982; Rai et al., 1997; Rai and Acharya, 2000). However, there is a compelling need to search for new antimicrobials from plants/oils. Taking into consideration the above fact, we report the antimicrobial activity of *Trigonella foenum-graecum* and *Pongamia pinnata* which are members of family Fabaceae. Traditionally,the seeds of *T. foenum-graecum* are used in dysentery, diarrhoea and inflammatory colic (Jean Brunaton, 1999) and as an antibacterial (Omolosa and Vagi, 2001). The seed-oil of *P. pinnata* is also medicinal and used in itches, abscess and other skin diseases (McCutchtchen et al., 1994; Khan and Evan, 1996; Kapoor, 2001).

MATERIALS AND METHODS

Extraction of oils

The healthy and mature seeds of *Trigonella foenum-graecum* and

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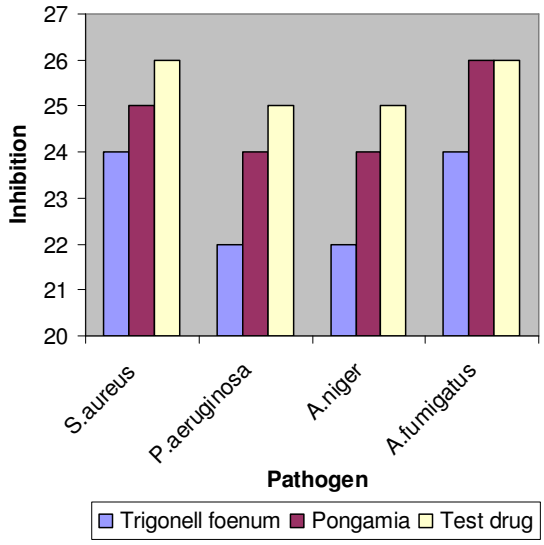

Figure 1. Antimicrobial activity of oils of *Pongamia pinnata* and *Trigonella foenum-graecum*.

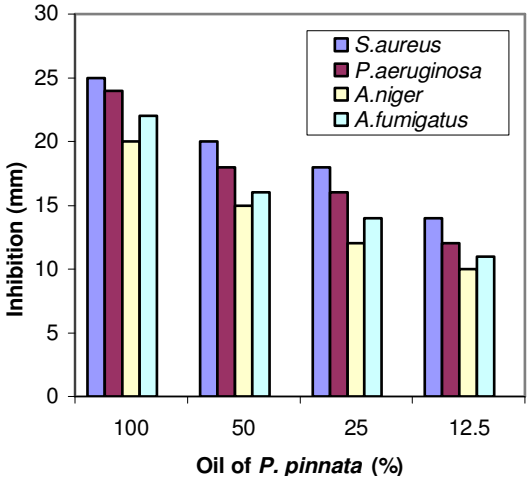

Figure 2. Antimicrobial activity of *Pongamia pinnata*

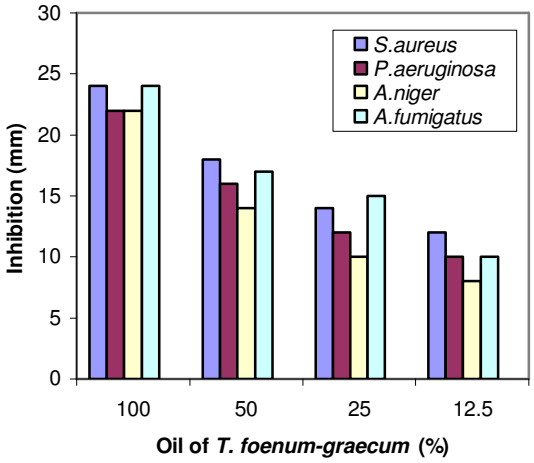

Figure 3. Antimicrobial activity of *T. foenum-graecum*.

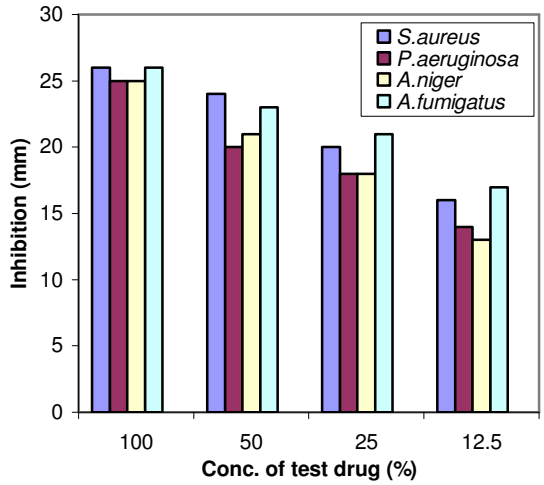

Figure 4. Antimicrobial activity of test drug.

Pongamia pinnata were collected from Amravati during 2003 and dried at room temperature and later powdered in grinder. About 15 g powder was used for aqueous extraction in Soxhlet. These oils were analyzed by GC/MS analysis.

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) analyses

The identification of volatile constituents from seeds was performed using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a HP-5971 mass selective detector and capillary column: HP-5 (30 m x 0.25 mm x 0.25 µm); oven temperature programme 60°C rising at 3°C min^{-1} to 240°C; injector temperature, 220°C, detector temperature, 250°C; split ratio, 1:30; carrier gas, helium; 1 mL min^{-1}. The GC-MS electron ionization system was set at 70 eV. A sample of the oil was methyled and solubilized in ethyl acetate for the analyses. Retention indices (RI) were determined by co-injection of hydrocarbon standards. The oil components were identified by comparison with data from literature (Adams, 2001), the profiles from the Wiley 138 and Nist 98 libraries, and by co-injection of authentic standards, when available. The quantification of the components was performed on the basis of their gas chromatography/flame ionization detector peaks on the HP-5 column

Test organisms

Aspergillus niger and *A. fumigatus*, two potential human pathogens, were obtained from Department of Biotechnology, Amravati University, Amravati. The cultures of these fungi were maintained on Czapeck Dox Agar at 28±2ºC. One-week old cultures were washed with sterile saline water and the spore suspensions were prepared by using glass wool filter. The colony forming units (cfu/ml) were determined and the test inocula were adjusted to 1.5 X 10^5 spores per ml. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were selected from the locally isolated human-pathogenic bacteria. The pure cultures of test bacteria were maintained on nutrient agar at 35ºC. After 24 h the cultures were used for experiment.

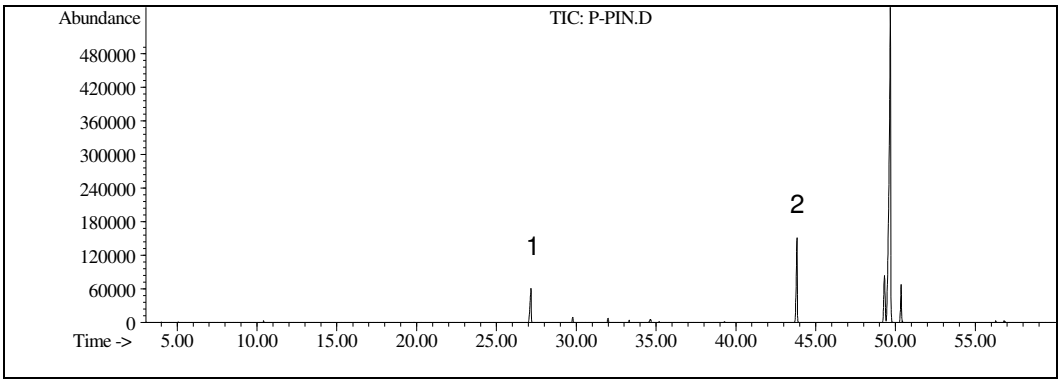

Figure 5. Chromatogram of *P. pinnata* oil.

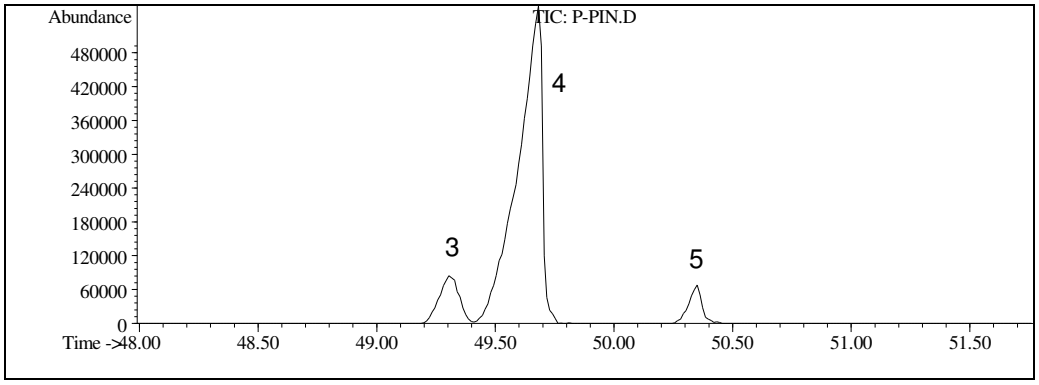

Figure 6. Section of chromatogram of *P. pinnata* oil (48–52 min)

Table 1. Compounds identified from the oil of *P. pinnata*

Peak	MW	Analyte	Fatty Acid	% [(a)]
1	186	Benzenesulfonic acid, 4-methyl-, methyl ester	metanilic acid	5.78
2	270	Hexadecanoic acid, methyl ester	palmitic acid	12.03
3	294	9,12-octadecadienoic acid, methyl ester	linoleic acid	8.51
4	296	9-octadecenoic acid, methyl ester	oleic acid	69.14
5	298	Octadecanoic acid, methyl ester	stearic acid	4.53

[(a)]Results expressed as % area.

Concentration of essential oils

Dimethyl sulphoxide (DMSO) was added in pure essential oils (100%) in order to obtain 50, 25, and 12.5% dilutions. Clotrimazole and amphicillin (1 mg/ml) were taken as control. Different dilutions of control were also prepared.

The paper disc technique

Petri dishes were filled with 10 ml of Czapeck Dox Agar for fungus and Nutrient Agar for bacteria in sterile conditions. One ml of the culture suspension was added per plate after the medium was solidified. Sterile discs (5 mm diameter, Whatman filter–paper No.42) were soaked in different concentrations of essential oils up to saturation. These saturated discs were placed at the center on agar surface, which were then incubated at 31ºC for 48 h. for fungi and for bacteria at 35ºC for 24 h. For each oil, triplicates were maintained. The clear inhibition zones were measured and noted.

RESULTS AND DISCUSSION

It is evident from Figure 1 that oils of *P. pinnata* and *T. foenum-graecum* showed strong inhibition against *S. aureus*, *P. aeruginosa, A. niger* and *A. fumigatus. P. pinnata* oil was more effective against *S. aureus* and *A. fumigatus.*

Both the oils were much effective after 24 h incubation against *S. aureus* and *P. aeruginosa*. This suggests that both the bacteria are more sensitive as compared to the fungi. These results corroborate the earlier findings of

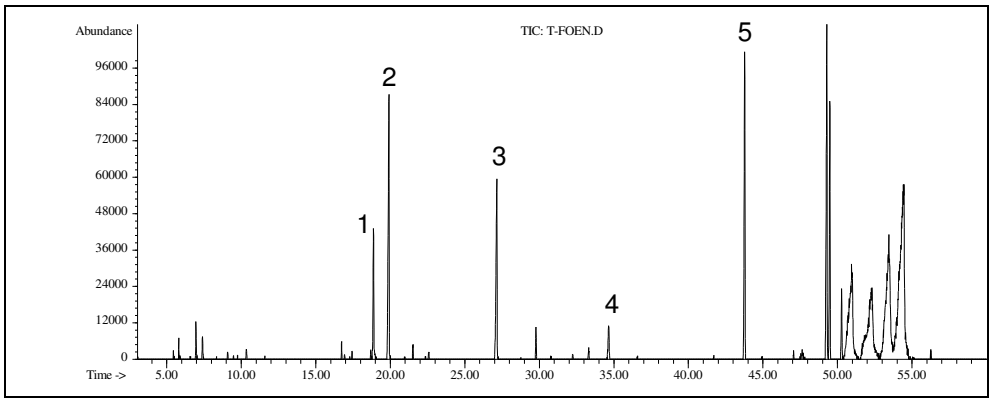

Figure 7. Chromatogram of *T. foenum graecum*.

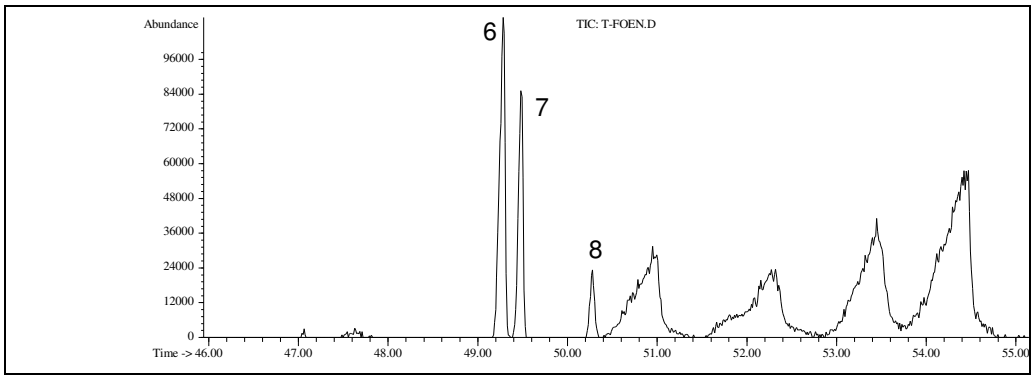

Figure 8. Section of chromatogram of *T. foenum graecum* (46–55 min).

Table 2. Compounds identified from the oil of *T. foenum graecum*.

Peak	MW	Analyte	Fatty Acid	% [(a)]
1	152	Not identified	-	7.97
2	152	Not identified	-	18.38
3	186	Benzenesulfonic acid, 4-methyl-, methyl ester	metalinic acid	14.24
4	---	Not identified	-	2.45
5	270	Hexadecanoic acid, methyl ester	palmitic acid	17.47
6	294	9,12-octadecadienoic acid, methyl ester	linoleic acid	21.48
7	296	9-octadecenoic acid, methyl ester	oleic acid	14.29
8	298	Octadecanoic acid, methyl ester	stearic acid	3.72

[(a)]Results expressed as % area.

Omoloso and Vagi (2001), who reported strong activity of *T. ƒoenum-graecum* against 26 bacterial pathogens. The maximum activity was recorded at 100%, whereas at 12.5% poor activity was noted. *P. pinnata* oil also showed the maximum activity against *S. aureus* and *P. aeruginosa* at 100% which must be due to active chemicals like pongarotene and or karanjin (Simin et al., 2002).

Both the oils were more effective against *A. niger* as compared to *A. fumigatus*. The above results revealed that the seeds oils can be effective antibiotic. These minimum inhibitory concentrations proved that the oils exhibited bacteriostatic activity at higher dilution factors.

Figures 5 and 6 and Table 1 show the chromatograms and compounds identified from *P. pinnata*, respectively. The chromatograms and compounds from *T. foenum graecum* are presented in Figures 7 and 8 and Table 2. The oil constituents were identified after samples methylation using the data sources available. The chromatographic analysis of oils obtained from the seeds of *T. foenum graecum* and *P. pinnata* showed saturated fatty acid, pal-

mitic and stearic, and the unsaturated acids, linoleic and oleic, besides metalinic acid (benzenesulfonic). Among these compounds, only the last is known as toxic and should be tested singly against the fungi in order to determine its inhibitory nature. Fatty oils of *P. pinnata* and *T. foenum-graecum* can be used as natural antimycotics after further studies. At the low concentrations, fatty oil of seeds of these plants showed very significant antimycotic activity against *A. niger* and *A. fumigatus*.

It can be concluded that fatty oils of these plants can be used for developing plant-derived antimicrobial drugs.

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